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DEVELOPMENTAL ANATOMY OF SEEDLING OF *JATROPHA CORDATA*¹

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The anatomical literature of the Euphorbiaceae is well summarized, up to 1902, by Gaucher (5) and, up to 1908, by Solereder (16). The more important papers which have appeared since 1908 include the work of Reiche (12), Swingle (19), Ferry (4), Helm (7, 8), Santos (14), Milanez (10), and Scott (15). Anatomical research done on the roots and stems of Euphorbes, reported in this literature, is principally concerned with the formation of laticiferous, tanniferous, and resiniferous cells or tubes; crystalliferous and granular cell inclusions; cork; collenchyma; fibers and sclerified cells; trichomes; and wood structure. Many problems, including the anatomy of the hypocotyl, studies on the time and place of tissue origin, and direction of tissue differentiation have been neglected. Apparently no one has investigated the entire stem, hypocotyl, and root anatomy of any species of the Euphorbiaceae.

MATERIAL AND METHODS

Jatropha is a large genus of the Euphorbiaceae closely related to *Hevea*, *Manihot*, and *Ricinus* (17) and is composed of about 150 species of trees, shrubs, and herbs. Its distribution is mostly tropical and subtropical in both hemispheres. In North America, *Jatropha cordata* (Orteg.) Muell. is found in Mexico on both sides of the Gulf of California, in the mountains of Baja California, and through Sonora, southern Chihuahua, and Sinaloa to Jalisco. When mature, this species has the growth form of a small tree 20-25 feet high with the superficial appearance of a shrub. A stem of this semi-woody plant 5 inches in diameter can easily be cut in two with one forceful slash of a machete.

Work was begun on the problem in the summer of 1939 at the Desert Laboratory of the Carnegie Institution of Washington at Tucson, Arizona. Seeds, collected near Hermosillo, Sonora, Mexico, were planted one-half inch below the soil level in pots in the greenhouse of the Desert Laboratory. Mature embryos, as well as seedlings of various ages (4, 7, 21, 41, and 75 days old), were preserved in Stover's fluid (3 cc. formalin, 30 cc. ethyl alcohol (95%), 67 cc. distilled water). During the summer, serial sections of the root, hypocotyl, and stem were cut and stained according to a safranin and fast green schedule modified from Johansen's (9).

GENERAL OBSERVATIONS

The root system of the seedling consists of one primary and four adventitious roots, all five of which are similar in size, anatomy, relative position, and time of origin. The primary root differentiates directly downward from the lower end of the hypocotyl. The four procambial strands of the hypocotyl coalesce about

¹Papers from the Department of Botany, The Ohio State University, No. 443.

50 μ above the collet, resulting in a core of pith and a cylinder of procambium which is continuous from the hypocotyl through the primary root. The four adventitious root primordia originate at the angles of an imaginary square, the corners of which are the points of junction of the four provascular strands of the hypocotyl.

During the first day or two after planting the seed, the adventitious roots remain imbedded in the cortex of the enlarged hypocotyl base (fig. 5). On the third day they push through the epidermis, and by the fourth day the adventitious roots and the primary root are all about 18 mm. long and equal in diameter. From this time on the five roots enlarge and elongate at about the same rate and are comparable in structure. The initiation of secondary roots does not occur until about the seventh day. These may appear first along the lower half of the five principal roots or nearer the base of the hypocotyl.

The straight hypocotyl of the embryo is about 2 mm. long. By the end of the fourth day after planting, when the cotyledons and endosperm emerge from the seed coat, the hypocotyl is about 18 mm. long. On the seventh day it reaches a maximum length of 10–13 cm., at which time the green cotyledons are free from the endosperm in which they were imbedded. The cotyledons bear the same relationship to the endosperm as in *Ricinus communis*. From the seventh day until the plant is two and one-half months old, the hypocotyl continues enlarging, especially at the basal end.

The hypocotyl of *Jatropha cordata* should not be considered a portion of the stem containing the transition region, but rather an organ of the plant possessing a developmental anatomy peculiar to it alone. In the stem-hypocotyl transition, the many leaf traces anastomose, forming four "synthetic bundles"² which alternate with the four cotyledon traces. In the upper one-half inch of the hypocotyl, the synthetic bundles anastomose with the cotyledon traces, resulting in four vascular bundles which extend through the organ (fig. 12) to within about 2 mm. of its base, where they coalesce.

The plumule is composed of one node, about 10 μ long, and the apical meristem which extends about 5 μ beyond it. No procambial strands are discernible. During the first four days, the stem elongates to about 120 μ and is composed of two nodes and the meristem which extends about 5 μ beyond the second node. One central and two lateral procambial strands become differentiated in each of the leaf primordia.

By the end of the first week, the stem is about 1 mm. long and six leaf primordia have become organized. The oldest primordium gradually becomes isolated from the five lying above it and from the cotyledonary node by the elongation of adjacent internodes. One new leaf primordium becomes differentiated each week until the plant is four months old or older. Consistently, the apical meristem extends 10 μ beyond the five youngest leaf primordia which remain closely aggregated in the first millimeter of the stem tip. As each successive primordium is organized, the oldest one becomes separated from the five above it by elongation of the intervening internode.

The procambium, which becomes organized some time within the first four days, is distinguishable as a complete ring of meristematic cells at the youngest node of the stem tip. The arc of procambial tissue extending down from the youngest leaf primordium makes up one-half the ring. Three provascular strands are observable in this arc as groups of smaller cells. The remainder of the procambial ring consists of an arc of small meristematic cells similar to and continuous with those of the apical meristem. At the time of differentiation, the cells of the ring show no radial seriation.

²As used by Priestley (11), a synthetic bundle is one "formed by the coalescence of the downward extension of the bundles from higher leaves."

Although there is some variation, especially in the first few internodes, most of the vascular bundles appear to extend through five internodes before anastomosing with other bundles.

In figures 1-4 the general pattern of growth by tissues in the root, hypocotyl, and stem has been recorded. From these graphs it may be inferred that the growth pattern is different for each of the three organs. A comparison of figures 2 and 3 indicates that the growth pattern is similar throughout the hypocotyl, except that growth is more than twice as rapid in the basal as in the upper end. An accurate interpretation of these graphs is dependent upon a knowledge of changes occurring in the various growing tissues. These observations are recorded in the following pages.

EPIDERMIS

The epidermis of the embryo is differentiated from a point about $370\ \mu$ from the root apex, through the hypocotyl, to within $10\ \mu$ of the stem apex. The thin-walled cells, dense with cytoplasm and radially elongated, divide only anticlinally. During the first four days the epidermal cells of the hypocotyl and most of the root vacuolate, elongate, and enlarge in the tangential direction. Concomitantly anticlinal divisions become less frequent. Cells of the hypocotyl frequently increase in length 4-6 times and double in cross sectional area. The same events occur in the stem as each successive internode elongates.

Ephemeral root hairs differentiate during the second or third day, about 9 mm. from the root apex. The nucleus frequently migrates into these protrusions, some of which become $225\ \mu$ long. By the end of the fourth day, many of these first-formed root hairs collapse and begin disintegrating.

A very thin cuticle covers the hypocotyl and plumule of the embryo. By the sixth week it forms a layer which varies in thickness from $1\ \mu$ at the stem tip and at the base of the hypocotyl to $6\ \mu$ in the mid portions of the shoot. At the same time that the cuticle is being formed, secondary epidermal cell walls form and become $1\ \mu$ thick by the middle of the third month.

Stomates differentiate in the epidermis of the embryo covering the basal third of the hypocotyl, and by the end of the third week they are present throughout the lower three-fourths of the epidermal cylinder. No stomates were observed in the epidermis of either the stem or the roots.

Sometime between the first and third weeks, the epidermis and some of the underlying layers of phellem begin splitting longitudinally in places throughout the lower 7 cm. of the hypocotyl. These breaks deepen and lengthen through the remainder of the hypocotyl, root, and stem as the organs increase in diameter. In the portions of the root one month old or older, the epidermis, hypodermis, cortical parenchyma, and outer layers of phellem have split (fig. 11). The crushed and disintegrating epidermal cells lie in broken sheets adhering to the ridges of bark. Sometime after the sixth week, the epidermis and the underlying layers of cork at the base of the stem begin splitting (fig. 13).

HYPODERMIS

The hypodermis of the embryo hypocotyl can be distinguished from the cortical parenchyma on the basis of the cell size, shape, contents, and staining properties but it does not differentiate in the root and plumule until about the fourth day. From that time on it is distinct throughout the plant from a point $10\ \mu$ from the stem apex to within $370\ \mu$ of the root apex. Although the tissue consists mostly of one layer of square or radially elongated cells, occasionally two and rarely more layers were observed in older roots.

Cessation of rapid anticlinal divisions soon after differentiation is followed by a three-day period of rapid vacuolation and tangential enlargement during which time the cells often triple in length.

In the root, during the first four days, the walls adjacent to the epidermis, the outer portion of the radial walls, and the collenchymatous thickenings which form between the epidermis and hypodermis undergo a chemical change, staining red with safranin. By the end of the first week the entire radial walls of many cells stain red. Collenchymatous thickenings begin forming in the intercellular spaces along the hypodermis-cortical parenchyma junction soon after the first week.

About the fourth day, periclinal divisions initiate a cork cambium in the hypodermis at the base of the hypocotyl. By the third week the cambium is present throughout the hypocotyl (fig. 12); after this time differentiation proceeds slowly, acropetally into the stem. When the plant is two and one-half months old, the hypodermis is uniseriate in the upper half of the stem while in the lower half there is one additional layer of periderm in each successively lower internode. At the collet there are eight layers and at the base of the hypocotyl, twelve layers. The rectangular, tangentially elongated cells of the periderm lie tightly compacted in radial seriation. Secondary cellulose walls, about $1\ \mu$ thick, are deposited inside the suberized primary walls of the phellem. The thin-walled cells of the phellogen are similar to those of the phellem in tangential dimension but only half as great in radial dimension. The phelloderm, which represents the enlarged centrad half of the original layer of hypodermis, is composed of one layer of cells comparable in size and vacuolation to those of the phellem but with thin, cellulose primary walls. After phellogen initiation, the cells of the phellogen and phelloderm slowly increase in tangential dimension except in the basal inch of the hypocotyl where the process is very rapid. When the cells are about double their original tangential dimension, they divide anticlinally. Consequently anticlinal divisions are most numerous in the basal inch of the hypocotyl.

CORTICAL PARENCHYMA

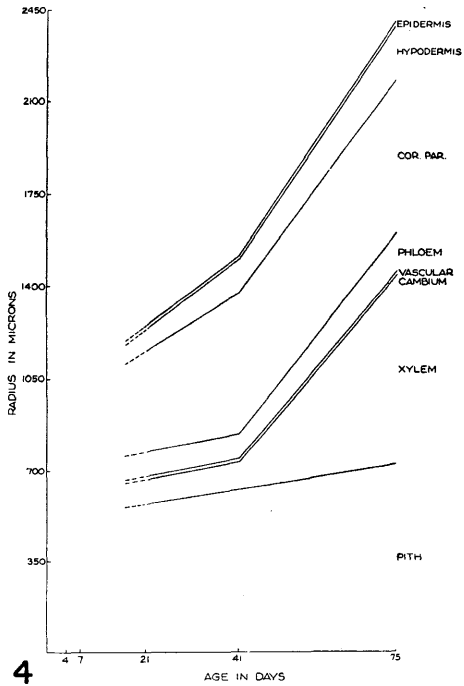
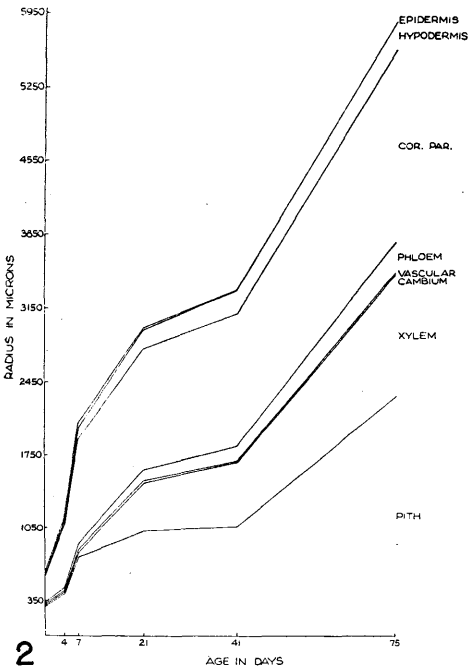
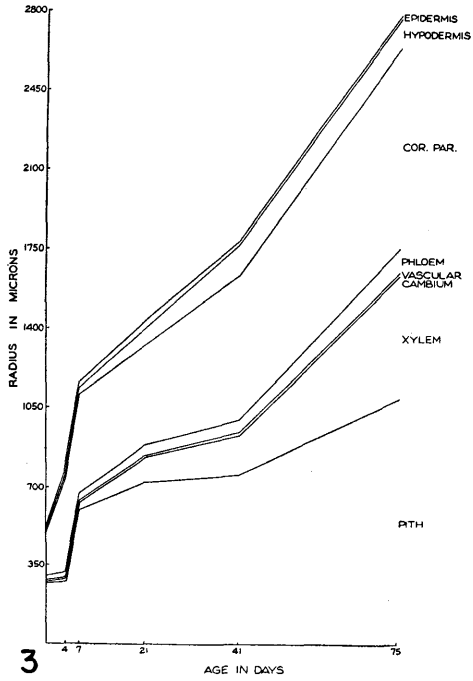
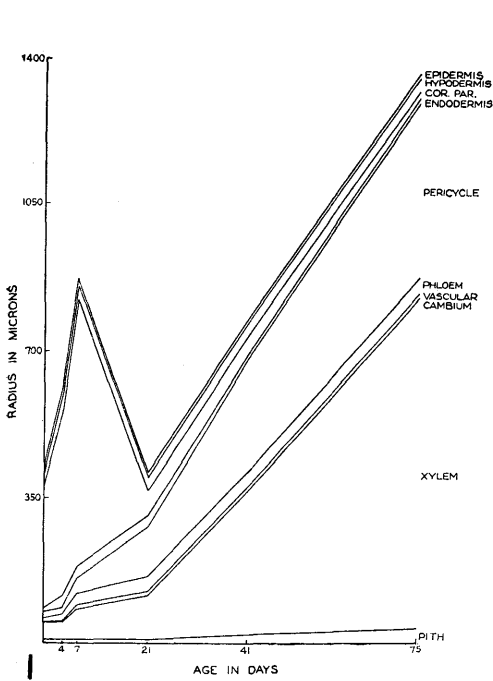
The cortical parenchyma of the embryo extends throughout the hypocotyl and to within $320\ \mu$ of the root apex. Strictly speaking, the tissue in the root has a definite inner boundary but an approximate outer one, since the endodermis is differentiated in the embryo stage but the hypodermis is not. In the root the number of cell layers (twelve) remain constant until disintegration of the tissue, except in the apical 1 mm. of the primordia where the number has not yet reached twelve. The cells of the eight inner layers are in radial seriation with the actively dividing endodermal cells (fig. 7). Periclinal as well as anticlinal divisions continue longest in those layers nearest the endodermis. Relatively large quantities of starch are present in the cells.

In the hypocotyl, anticlinal and periclinal divisions are restricted to areas adjacent to the phloem for the first 2-3 weeks, after which they spread radially through the cortical parenchyma in the basal inch of the organ and then gradually, acropetally. Divisions are at all times more numerous in the lower inch of the hypocotyl.

During the first week the cells of the cortical parenchyma in the root primordia and hypocotyl undergo tremendous enlargement at which time they lose their decided tangential elongation and become more or less circular in cross section.

EXPLANATION OF PLATE I

- FIG. 1. Graph showing relative widths of the various tissues along the radius during differentiation and maturation in the oldest portion of the root.
FIG. 2. Graph showing relative widths of the various tissues along the radius during differentiation and maturation in the basal end of the hypocotyl.
FIG. 3. Graph showing relative widths of the various tissues along the radius during differentiation and maturation in the top end of the hypocotyl.
FIG. 4. Graph showing relative widths of the various tissues along the radius during differentiation and maturation in the oldest portion of the stem.



Many cells, especially in the hypocotyl, increase in cross sectional area 10-15 times and in length 1-4 times.

Between the first and third weeks, the cortical parenchyma in the oldest portions of the root undergoes a collapse, and cell disintegration begins (fig. 10). The radial dimension of the tissue decreases about 80 per cent during this two-week period, and by the middle of the third month only fragmentary remnants of the disintegration products of the protoplasm and cell walls remain (fig. 11).

A few larger, thicker-walled, mostly empty cells which become filled with latex during the first 3-4 days, differentiate adjacent to the phloem in the hypocotyl; they appear in the stem soon after differentiation of the cortical parenchyma. The cortical parenchyma of the stem becomes differentiated at the youngest node, about 10 μ from the stem apex. The small cells are tightly compacted and divide in all planes, although most frequently periclinally. At the time of internode elongation, the cortical parenchyma of the stem becomes distinctly divided into two concentric rings of tissue (fig. 13). Divisions cease in the outer layers of cells, which begin enlarging and elongating tangentially, but centrad to this outer ring and interspersed among the enlarging parenchyma and latex cells, strands of small, dividing cells persist. Similar groups of dividing cells appear in the hypocotyl at about the end of the first week. During the second week, beginning at the base of the hypocotyl and proceeding acropetally, the larger, scattered cells in these strands begin forming secondary cellulose walls. Differentiation of fibers from parenchyma cells proceeds acropetally through the hypocotyl and stem at a rapid rate. Accompanying the differentiation of fibers is an increase in the number and thickness of the laminations deposited inside their primary walls. By the middle of the third month, the secondary walls of fibers in the basal inch of the hypocotyl are about 11 μ thick while in the rest of the hypocotyl and older portions of the stem, they are 7-8 μ thick.

The most recently differentiated fibers in the lower inch of the two and one-half months old hypocotyl are mostly two or more times larger in cross sectional area than any in the shoot. Beginning with the origin of the fiber initials, the enlargement and division of the intervening parenchyma cells results in further separation of the groups of fibers from the phloem. Since the rapidity of cell division and the degree of cell enlargement are greater in the basal inch of the hypocotyl, cortical fiber groups here become farthest separated from the phloem.

Chloroplasts appear in the cortical parenchyma of the hypocotyl and stem during the period of rapid cell enlargement. They are most abundant in the cells nearest the hypodermis. During the week following rapid internode elongation, collenchymatous thickenings are initiated in the two or three layers of tightly compacted cells of the stem adjacent to the hypodermis.

ENDODERMIS OF ROOT³

In the embryo, the endodermis forms a cylinder of tangentially elongated cells lying immediately external to the pericycle and extending from the collet to within about 320 μ of the apex of the root primordia (fig. 7). The frequent periclinal divisions and occasional anticlinal ones contribute new cells to the cortical parenchyma and endodermis, respectively (fig. 7). Occasionally the endodermis becomes more than one cell thick in places.

Small, intercellular spaces originate during the first four days between the endodermis and the cells of the adjoining tissues. Simultaneously Casparian strips are initiated. Although the evidence at hand is insufficient to warrant a positive statement, it seems probable that these thickenings originate on the cell walls adjacent to the intercellular spaces between the endodermis and pericycle and gradually move out the radial walls to about the mid-point.⁴ For a few days the Casparian strips become more prominent, but in the second or third week,

³No endodermis differentiates in either the stem or hypocotyl.

⁴These observations seem to be in accord with the findings of D. S. Van Fleet (20).

when the walls of the endodermal cells undergo a chemical change and stain red with safranin, they lose their identity. Collenchymatous thickenings which stain red with safranin occasionally fill the intercellular spaces adjacent to the endodermis. Beginning about the third week and accompanying disintegration of the cortical parenchyma, the endodermis splits and becomes disorganized (fig. 10). By the middle of the third month it appears fragmentary and mostly crushed to extinction in the ridges of bark.

PERICYCLE OF ROOT⁵

In the embryo root primordia, the pericycle is differentiated to within 320 μ of the apex as a cylinder of cells bounded on the outside by the endodermis and centrad by much smaller cells of the stele (fig. 7). Anticlinal and periclinal divisions in the pericycle contribute cells respectively to a cylinder of pericycle one cell wide and to two strands of small, dividing cells appearing crescent-shape in cross-section. These strands of pericyclic cells lie on each side of the central axis of the diarch root, with the convex arc adjacent to the cylinder of pericyclic cells. (Cf. pericyclic fiber region (PF) in figs. 7, 9, 10.) During the first four days cells nearest the primary xylem points, in the narrowest parts of the strands, begin vacuolating, and some of their walls begin thickening. By the end of the third week all these closely compacted, dividing cells have enlarged and many have differentiated as fibers with thick, laminated secondary walls (fig. 10). Enlargement and division of the parenchymatous cells within the strands, together with pressures exerted by the increasing amount of secondary xylem and phloem, result in a scattering of the pericyclic fibers within the strands and the further separation of the two fiber groups.

During the latter part of the first week the cells of the pericyclic cylinder enlarge slightly in tangential direction, and concomitantly cells opposite the diarch xylem points in the oldest part of the root begin dividing periclinally. From these two original locations, periclinal cell divisions spread tangentially and acropetally through the pericyclic cylinder. By the end of the third week this pericyclic cambium (phellogen) is complete to a point about 400 μ from the root apex (fig. 10). Both phelloderm and phellem are formed, while the phellogen remains as the middle layer (figs. 10, 11). The thin walls of the loosely compacted phelloderm cells are mostly cellulose, while the tightly compacted cells of the phellem have thick, suberized walls. Toward the end of the first week, secondary root primordia are initiated in the pericycle opposite the points of the diarch xylem. All primordia observed were 3.5 mm. or farther back of the root apex.

PHLOEM

Differentiation is very difficult to observe in the phloem, and therefore exact boundaries of this tissue cannot always be accurately determined. Protophloem and metaphloem are indistinguishable.

In the embryo four strands of small, angular, dividing phloem cells extend through the hypocotyl and to within 380 μ of the root apex. In the hypocotyl they lie opposite the four xylem strands while in the root they lie on each side of the xylem points adjacent to the pericycle (fig. 7). Similar primary phloem groups differentiate out of the procambial strands at the fourth node, about 50 μ from the apex. In the root and hypocotyl during the first week and in the stem at the time of internode elongation, intercalary cell divisions become less frequent and the cells increase in length and cross-sectional area 2-5 times. The most frequent divisions and the most extreme enlargement of cells occurs in the basal inch of the hypocotyl. Soon after cell enlargement, sieve tubes, companion cells, and phloem parenchyma differentiate.

Secondary fascicular phloem begins forming in the roots within the first four

⁵No pericycle differentiates in either the stem or the hypocotyl.

days and in the hypocotyl and stem toward the end of the first week. Slow differentiation of secondary interfascicular phloem begins several days following the acropetal differentiation of the interfascicular cambium which is initiated at the base of the hypocotyl during the latter half of the first week. About the second or third week, external to the small portions of secondary vascular cambium, opposite the diarch xylem points in the root, tertiary phloem is initiated. Secondary and tertiary phloem cells are usually larger than those of the primary phloem and lie in radial seriation.

In the second or third week phloem fibers, resembling those of the cortical parenchyma, become differentiated in the basal inch of the hypocotyl. Differentiation proceeds slowly, acropetally through the hypocotyl and into the stem about the end of the second month. They begin differentiating in the root about the middle of the sixth week.

If the cross-sectional area of the companion cells of a $2\frac{1}{2}$ months-old root is arbitrarily used as one unit, the sieve tubes are $2\frac{1}{2}$ units in area, the fibers 5.5 units, and the phloem ray cells 11 units.

Owing to the greater number and size of the parenchyma cells lying between the phloem and the fibers of the cortical parenchyma, phloem fibers may be much more readily distinguished in the lower inch of the hypocotyl than in the upper part. In general, phloem fibers are significantly smaller than fibers of the cortical parenchyma.

VASCULAR CAMBIUM

The fascicular cambium of the embryo extends through the hypocotyl only and appears to have differentiated basipetally. These cambial initials elongate tangentially and soon double or triple in that dimension. In the latter half of the first week, concomitant with the elongation of the first internode of the stem and the origin of the phellogen of the hypocotyl and root, (1) fascicular cambia originate at the hypocotyl end of the root, (2) interfascicular cambia originate in the basal end of the hypocotyl, and (3) fascicular cambia originate in the stem.

Differentiation of the fascicular cambium of the stem proceeds basipetally from the third node (about $20\ \mu$) from the stem apex. Cambial cells initiating xylem vessel elements and tracheids are mostly three times greater in tangential dimension than those initiating xylem parenchyma. Immediately following initiation of fascicular cambia, which occurs quickly, parenchyma cells adjacent to the ends of the fascicular cambia divide periclinally and by the time internode elongation is complete, the tangential extension of the interfascicular cambia is complete. Differentiation proceeds acropetally.

After the initiation of the interfascicular cambia at the ends of the fascicular cambia in the base of the hypocotyl, differentiation proceeds laterally and acropetally. By the second or third week the vascular cambium forms a ring in the basal inch of the hypocotyl and by the sixth week it has become a cylinder extending the length of the organ. The vascular cambium continues to be more active in the basal inch than elsewhere in the hypocotyl.

The interfascicular cambial initials in the stem and the basal inch of the hypocotyl increase in size very rapidly but upon becoming twice or three times as great in tangential dimension as the fascicular cambial cells, they divide anticlinally.

EXPLANATION OF ABBREVIATIONS

USED IN FIGURES 7, 9, 10 AND 11

(EP, epidermis; H, hypodermis; CO, cortex; EN, endodermis; PER, pericycle; PC, phellogen originating from the pericycle; PF, pericycle fibers or profiber strands; PH, phloem; CA, vascular cambium; CA₂, secondary vascular cambium; SX, secondary xylem; TX, tertiary xylem; XR, xylem differentiated from phelloderm cells formed by the pericyclic (cork) cambium.)

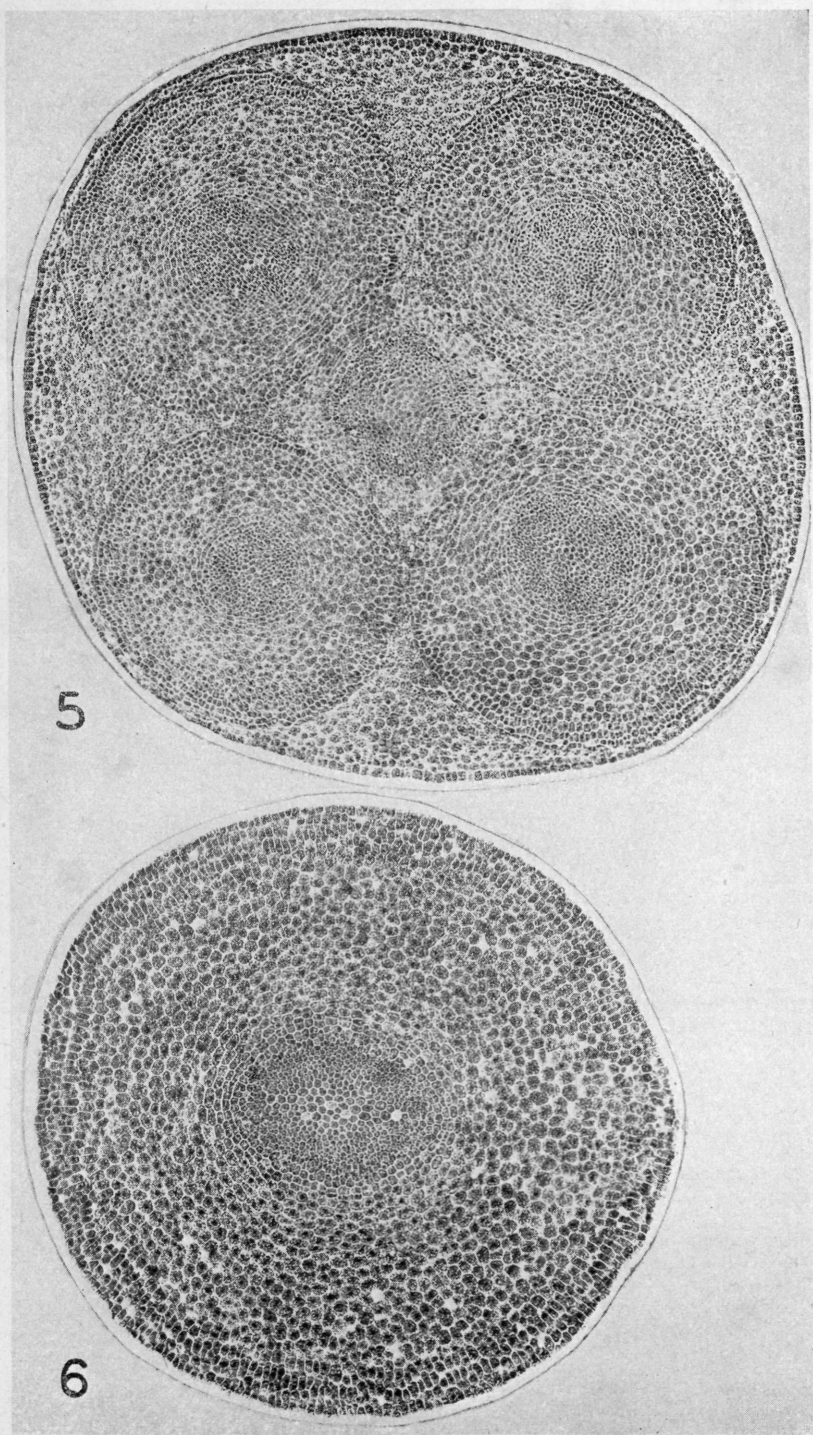


FIG. 5. Cross-section through the basal end of an embryo hypocotyl with cross-sections of a primary and four adventitious roots. $\times 94$

FIG. 6. Cross section through the oldest portion of an embryo primary root. $\times 45$

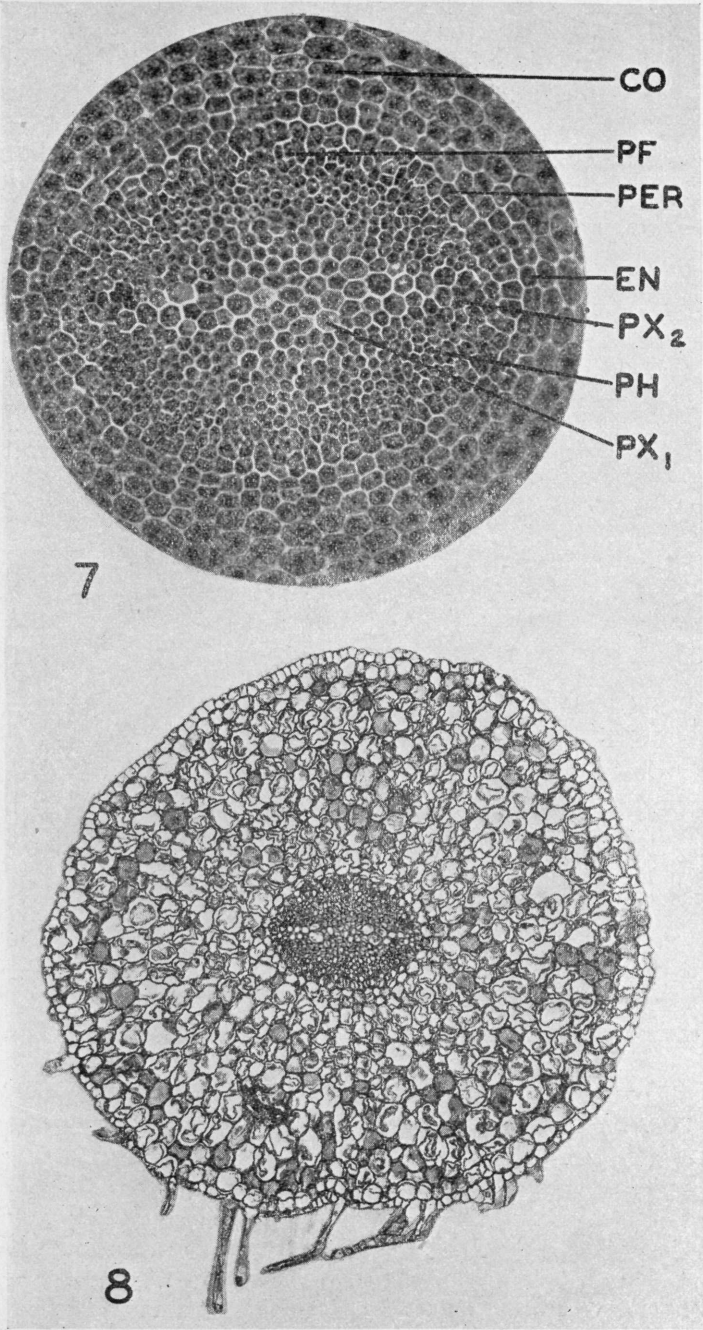


FIG. 7. Cross-section through the central part of the oldest portion of an embryo primary root. $\times 205$
FIG. 8. Cross-section through the oldest portion of a root four days old. $\times 110$

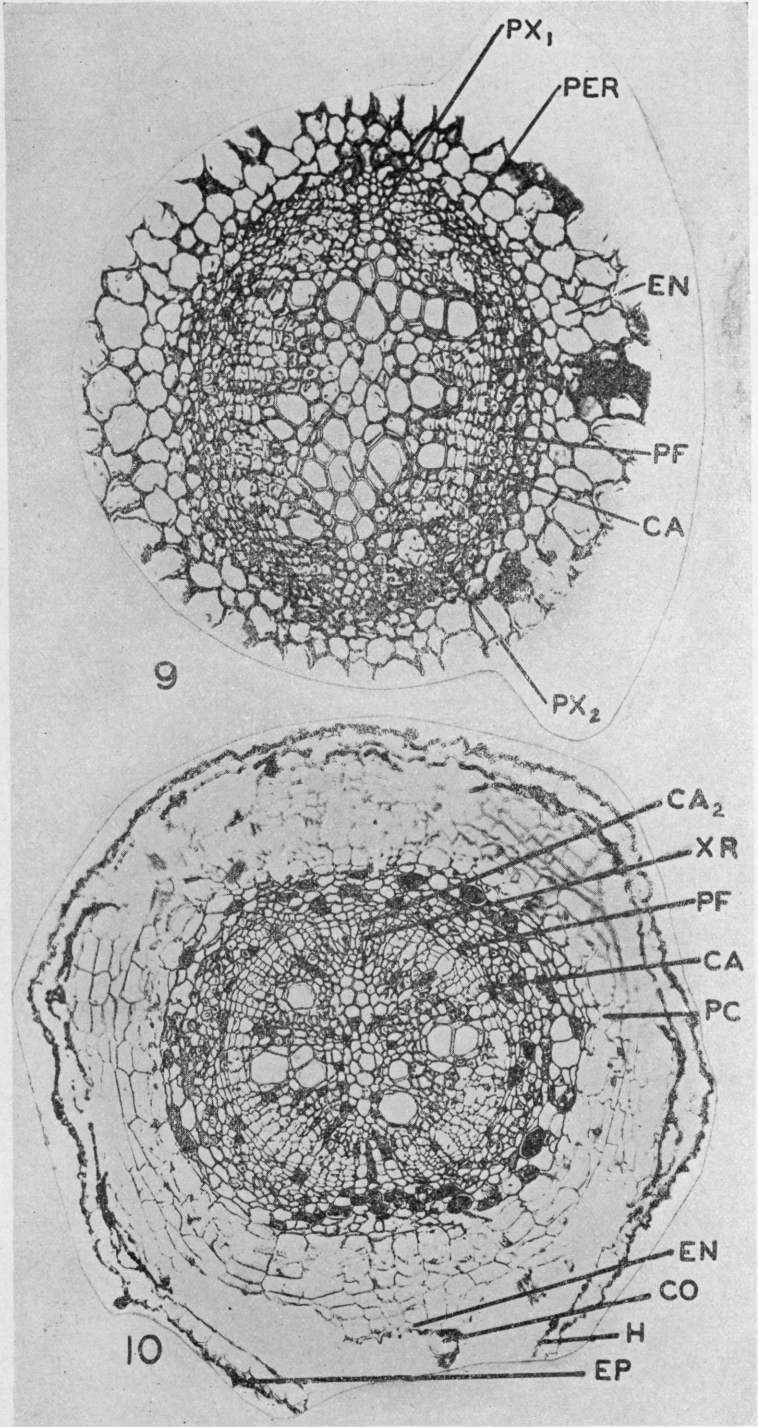


FIG. 9. Cross-section through the central part of the oldest portion of a root seven days old. $\times 175$

FIG. 10. Cross-section through the oldest portion of a root three weeks old. $\times 121$

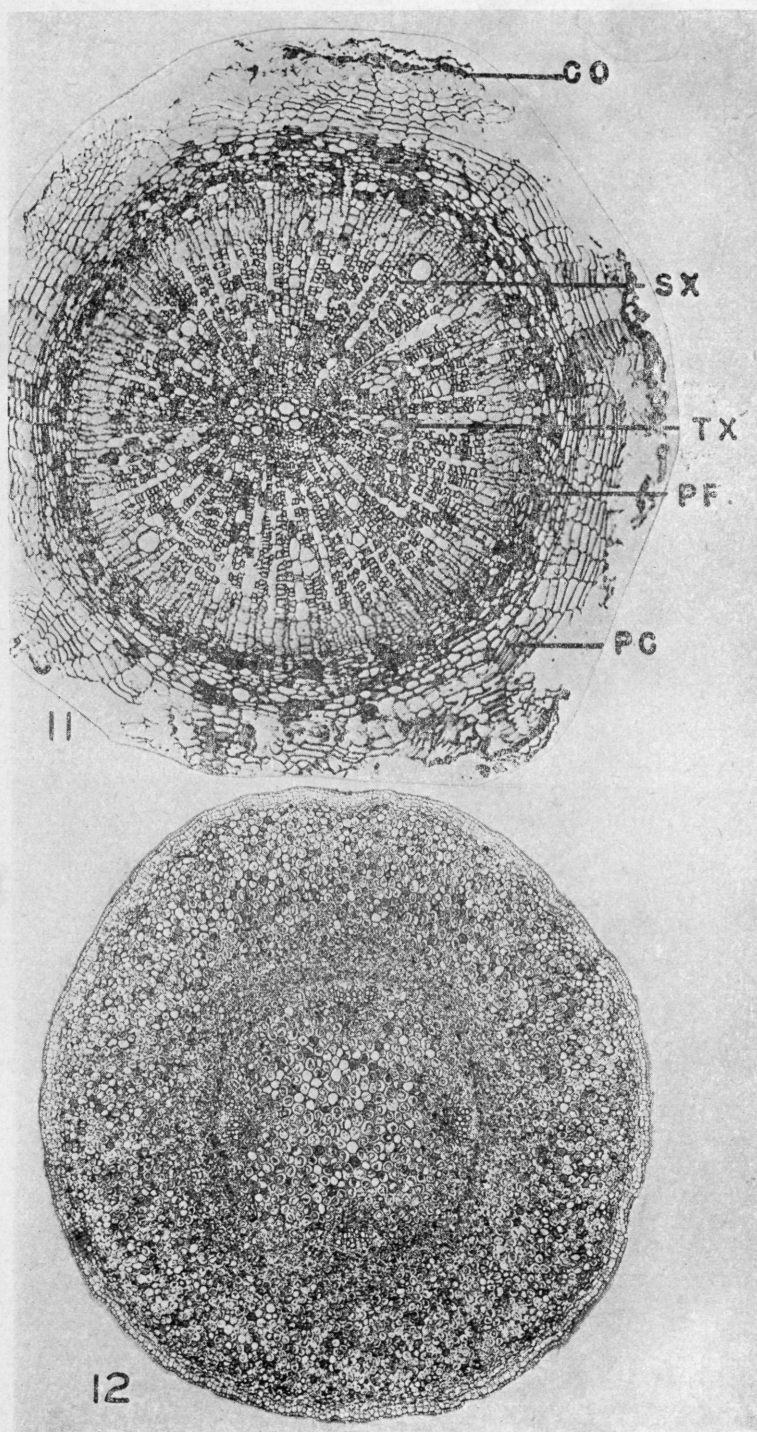


FIG. 11. Cross-section through the oldest portion of a root two and one-half months old. $\times 48$
FIG. 12. Cross-section through the swollen basal end of a hypocotyl seven days old. $\times 21$

The vacuolated initials in the root first appear in four positions along each side of the diarch xylem points but separated from them by xylem parenchyma. The formation of cambial initials proceeds laterally and acropetally until about the end of the first week when the primary vascular cambium is composed of two sheets of cells lying in arcs on each side of the xylem axis (fig. 9). By the end of the third week, and from that time on, they extend to within 1.6 mm. of the root apex. As the xylem increases in bulk, the tangential dimension of the cambial cells doubles or triples. Those initiating xylem or phloem ray parenchyma cells become twice as great in radial and tangential dimensions as other cambial cells. Anticlinal divisions are few.

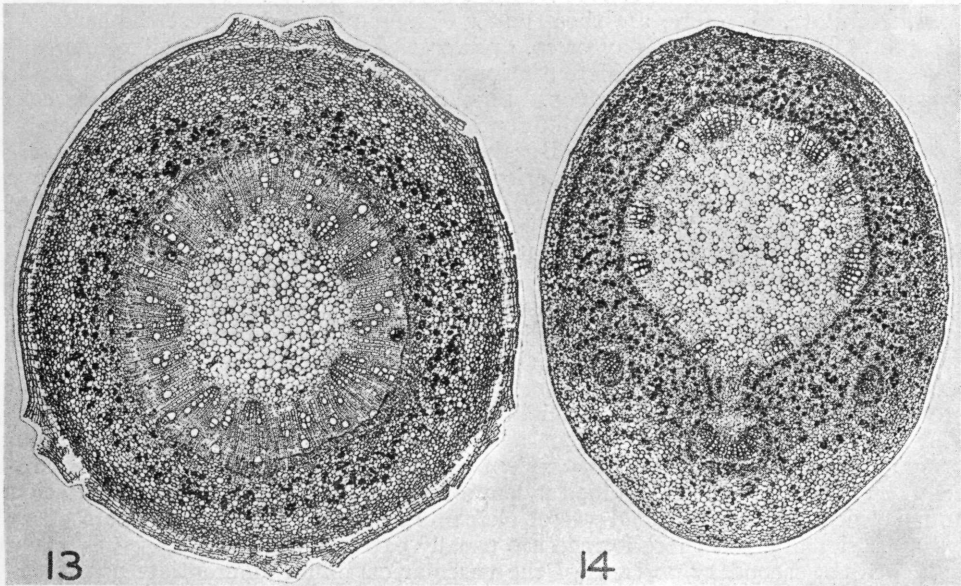


FIG. 13. Cross-section taken immediately above the second node of a stem two and one-half months old. $\times 23$

FIG. 14. Cross-section taken immediately below the seventh node of a stem two and one-half months old. $\times 17$

Most of the secondary tissue which originates from the vascular cambium is xylem (fig. 9), and although the amount of it becomes constantly greater on each side of the central axis, none is formed opposite the xylem points because the vascular cambium has not yet differentiated there (fig. 9). These gaps in the xylem, fan-shaped in cross-section and opposite the diarch xylem points, are in the meantime being filled with phelloderm cells originating from the pericyclic (cork) cambium. The cells are slightly larger than those of the xylem, and many of them are tangentially elongated. Soon after these wedge-shaped groups of phelloderm cells originate, the vascular cambium begins differentiating across them at the hypocotyl end of the root (fig. 10). The differentiation of these secondary portions of the cambial cylinder proceeds so slowly, acropetally, that by the end of the third month they are found only through the upper one-fifth (3 cm.) of the root. The six or seven layers of phelloderm lying centrad of the newly differentiated secondary portions of the vascular cambium become differentiated as xylem (fig. 10).

Sometime after the sixth week, external to the secondary portions of the vascular cambium, phloem becomes differentiated from phelloderm cells, and very soon pericyclic fibers differentiate external to the phloem. In the oldest portion of a $2\frac{1}{2}$ month-old root, the pericyclic fibers form a complete cylinder (fig. 11).

XYLEM

Primary xylem is present in the root but is never found either in the stem or in the hypocotyl, with one minor exception.

A primary diarch xylem is defined in the steles of the embryo root primordia to within $310\ \mu$ of the apex, on the basis of cell size and position (figs. 6, 7). The xylem cells nearest the center of the root stop dividing and become greatly enlarged before divisions cease in the cells nearest the pericycle (fig. 7). The first xylem cells to differentiate, therefore, are those larger elements located nearest the center of the root. Within the first day or so, proliferation ceases in the small xylem cells adjacent to the pericycle. Differentiation proceeds from the hypocotyl end of the root to within $1,000\ \mu$ of the apex. Lignification and maturation of these cells begins the next day following differentiation and progress rapidly acropetally but rather slowly centripetally. By the end of the fourth day approximately thirty of the elements nearest the pericycle at the hypocotyl end of the root have spiral or annular lignified secondary walls (fig. 8). It is not until about the end of the first week that secondary wall formation and lignification begins in those cells nearest the center of the root. The lignified walls of these cells, which frequently become $3\ \mu$ thick, are reticulate or occasionally pitted.

Simultaneous with the formation of the two arcs of vascular cambium, xylem fibers become differentiated from primary cells on opposite sides of the diarch xylem axis. The xylem fibers are similar to the pericyclic and phloem fibers, except that on the average they are two or three times larger in cross-sectional area. Their secondary walls are slightly lignified, often becoming $3\ \mu$ thick but they frequently contain cytoplasm and nuclei. Differentiation of fibers from primary and then from secondary xylem parenchyma proceeds rapidly, centrifugally, on both sides of the lignified xylem axis (figs. 8, 9, 10, 11). Differentiation and lignification of additional vessel elements scattered among the fibers occur only occasionally, but those formed are usually extremely large.

When the secondary portions of the vascular cambium differentiate across the wedges of secondary tissue opposite the xylem points (fig. 10), the cells lying centrad of them differentiate as xylem fibers, parenchyma, and lignified elements. Cells initiated centripetally by the secondary portions of cambium become differentiated as tertiary xylem (fig. 11).

Uniseriate or biseriate rays of xylem parenchyma begin differentiating during the fifth or sixth week. These large, thin-walled cells are sometimes crushed by the enlarging elements adjacent to them. In the oldest part of a root $2\frac{1}{2}$ months old, lignified elements, very few of which are located in tertiary tissue, comprise about 10 per cent of the xylem bulk. Xylem parenchyma, most of which lies in xylem rays and close to the cambium, makes up about 20 per cent of the xylem. The remaining 70 per cent consists of fibers (fig. 11). The largest cells of each type are those most recently initiated by the cambium. This is partly due to the gradual increase in size of the vascular cambium initials.

The secondary xylem of the embryo hypocotyl lies in four equally spaced strands which are continuations of the cotyledonary bundles (fig. 12). Each xylem strand contains about five radial rows of enlarged cells which later mature as vessels. Each of the rows is separated by one or more rows of xylem parenchyma. Since there are five vessel initials per row in the basal end of the hypocotyl and six at the top end, it may be inferred that differentiation of the vascular cambium and its secondary xylem proceeded basipetally. Maturation, indicated by lignification of secondary thickenings and disintegration of cell contents, follows quickly,

centrifugally during the first four days. On the third or fourth day the maximum number of vessels per row is reached, there being about eight per row in the basal inch and six per row in the rest of the hypocotyl. In the first lignified vessels the thickenings are annular, and in successively younger elements, stretched spirals, spirals, and tight spirals were observed. Some of the cells of the xylem parenchyma lying between the rows of vessels enlarge greatly and become differentiated as uniseriate, or occasionally biseriate, xylem rays which are perpetuated by divisions of certain cambial cells. Most of the cells differentiate as xylem fibers possessing slightly lignified, cellulose, secondary walls which frequently become $3\ \mu$ thick. At this same time, several very small cells with annular, partly lignified thickenings become differentiated from the primary pith parenchyma at the secondary xylem points.

Between the fourth and seventh days xylem cells are initiated by the interfascicular cambium in the basal inch of the hypocotyl and rapidly enlarge until they are nearly comparable in cross-sectional area to the largest vessels. Additional cells are formed as the interfascicular cambium differentiates laterally and acropetally. The first five layers of cells produced before the end of the third week remain parenchymatous. Many of the cells initiated between the third and sixth weeks in the basal end of the hypocotyl differentiate as fibers. Among these are scattered a few vessels whose secondary walls become thick and lignified. Sometimes after the sixth week rays of large parenchyma cells differentiate in the interfascicular xylem.

The secondary xylem of the stem originates at the third node, about $20\ \mu$ from the stem apex with the differentiation of one small strand of cells representing the downward extension of the central vascular bundle of the leaf primordium attached at that node. At the time of internode elongation twelve leaf traces are differentiated (fig. 14). In each trace there are about five uniseriate plates of xylem parenchyma alternating with four uniseriate plates of vessels. Vessels continue differentiating until about the sixth week, when the maximum number (five to nine) per radial row has been reached. In the second or third week xylem fibers begin differentiating from newly formed secondary xylem parenchyma, and by the middle of the third month the secondary cellulose walls are $1\ \mu$ thick.

Vessel differentiation in the leaf traces is basipetal through the stem, as evidenced by the decrease in vessel number, secondary wall thickness, and secondary wall lignification. Initiation and lignification of vessels occur first in the larger central trace of the leaf, and later in the two lateral traces.

During the second week the interfascicular cambia of the stem begin differentiating acropetally and tangentially from the ends of the fascicular cambia. The first few layers of xylem cells, those lying farthest centrad remain parenchymatous, but most of those cells originating from the interfascicular cambia after the third week differentiate as fibers. Occasional vessels differentiate among the fibers of both the fascicular and interfascicular xylem (fig. 13). Beginning about the sixth week uniseriate rays composed of large parenchymatous cells differentiate throughout the xylem. Occasionally the walls of these cells become slightly thickened.

PITH

Pith is the first tissue of the root to differentiate. Its closely compacted, angular cells extend to within $300\ \mu$ of the root apex and form a continuation of the pith of the hypocotyl. The large cells usually remain parenchymatous but occasionally the walls become slightly thickened.

In the embryo hypocotyl the cells are loosely compacted, dense with cytoplasm, and contain much starch, most of which disappears during the third week. Anticlinal and periclinal divisions occur frequently only in the lower end of the hypocotyl. Divisions cease during the first four days but are reinitiated in the

fourth or fifth week. The cells begin enlarging soon after their formation, and by the third week those in the base of the hypocotyl have increased in cross-sectional area more than seven times, those in the upper parts more than two times. Cell elongation occurs concomitantly and is approximately equal in the cells throughout the organ. On about the seventh day a cavity formed by the tearing of cells in the center of the pith originates in the base of the hypocotyl, and by the third week it extends throughout the organ.

Pith becomes differentiated in the stem at about the fourth node, 60 μ from the apex. The cells are closely compacted and contain no starch. Cell divisions occurring in all planes cease at about the time of internode elongation. The cells gradually become rounded as they increase greatly in size.

DRUSES AND LATICIFEROUS CELLS

Large, spherical aggregates of crystals (druses), presumably of calcium oxalate, and appearing yellow in prepared sections, were observed filling isolated cells in many tissues of the plant. They first appear in the cortical parenchyma of the root on about the seventh day; in the tissue lying centrad of the pericyclic (cork) cambium in the second or third week; and in the phloem, especially in the phloem rays, sometime after the sixth week. In the hypocotyl they first appear in the cortical parenchyma and pith between the fourth and seventh days; in the phloem and hypodermis between the first and third weeks; and in the xylem sometime after the sixth week. In the stem they first occur in the hypodermis, cortical parenchyma, phloem, and pith in the second or third week.

Laticiferous cells occur singly, or form short crooked tubes in many tissues of the plant. The contents of these cells stain red with safranin. In the root, laticiferous cells differentiate in the xylem, phloem, and tissue centrad of the pericyclic (cork) cambium, during the second or third week. In the hypocotyl they differentiate in the cortical parenchyma of the embryo; in the phloem during the fifth or sixth day; and in the xylem between the first and third weeks. Both druses and laticiferous cells are more abundant in the basal inch of the hypocotyl than in the upper parts of the organ. In the stem they differentiate among the cells of the meristem in the embryo stage. After tissue differentiation, they are found in the cortical parenchyma. Latex frequently fills the lignified vessels in the stem.

TERMINOLOGY

In this paper, a cell or tissue is considered *differentiated* from a group of cells when it can be distinguished from them on the basis of differences in cell size, shape, contents, rate of cell division, or differences in the cell wall. It has been assumed that the cell or tissue continues differentiating as long as visible⁶ changes occur in it.

The term *mature* has been applied to cells which are incapable of further differentiation, that is, dead cells. The writer is aware that cells may become "physiologically mature" long before death but—since it is impossible to determine whether the processes in a cell will undergo considerable change later in life or remain more or less constant—it is impossible to determine when a cell is "physiologically mature."

In recording the observations on xylem, it will be noted that use of the terms *proto-* and *metaxylem* has been avoided. So many misconceptions regarding these terms are at large in the literature that they no longer convey the same meaning to all plant anatomists. It would seem, therefore, that these concepts are in urgent need of re-examination and revision in the light of recent facts.

The terms were proposed by Russow (13) in 1872. As defined by him,

⁶It is understood that physiological differentiation precedes visible differentiation and that it may continue afterward.

protoxylem refers to the first xylem elements to *differentiate*. He states not only that these elements are primary in origin, but that they are the first to become thick walled and hence to mature. He realized, however, that the pattern of the secondary wall is directly related to the degree and speed of cell elongation at the time of secondary wall formation.

DeBary (2) apparently retained the term as defined by Russow but imposed one additional limitation by stating that protoxylem consists of tracheae with spiral or annular thickenings.

According to Coulter, Barnes, and Cowles (1), "The first xylem elements to appear (in the stem) are small in caliber, and of the spiral kind, a kind especially adapted to a region of rapid elongation. These groups of spiral vessels are called the *protoxylem*, and the later vascular elements form the *metaxylem*."

In Eames and MacDaniels (3), "The first cells of the phloem to mature are known as the *protophloem*, those of the xylem, the *protoxylem*." The cells of the protoxylem are further characterized as being primary in origin, narrow and slender, and possessing annular, spiral, or perhaps scalariform secondary walls.

Hayward (6) states that "The primary xylem is further divided into the *protoxylem*, which is the first to differentiate and mature at a given locus, and the *metaxylem*, which usually matures later." Elsewhere he defines protoxylem as "the first elements of primary xylem to be differentiated, characterized by annular, spiral, or reticulate secondary walls."

Priestly and Scott (11) have showed that the xylem of *Helianthus annuus* stem is all secondary in origin. The first xylem elements to differentiate are also the first to mature and possess annular or spiral secondary walls. Those elements differentiating and maturing later are characterized by scalariform, reticulate, and pitted secondary cell walls. In the roots of *Helianthus*, the first xylem formed is primary in origin. These workers offer the suggestion that "It would seem far better to use the terms 'primary' and 'secondary' with their usual implications and to recognize them in the shoot, . . . the protoxylem is usually secondary, being derived from the inner members of the radial series of cells cut off from the cambium." Since protoxylem and metaxylem have in the past connoted primary origin to most of us, it would seem that although this suggested usage of the terms is fundamentally sound, general acceptance and accomplishment of such a change would be a practical impossibility and would only result in further confusion in the literature.

Stover (18) has called attention to the absence of xylem elements with annular or spiral secondary walls in the slow-growing rhizomes of *Agropyron repens*, *Spartina michauxiana*, and *Calamovilfa longifolia*. In the same paper he also reports that in the vascular bundles of rapidly growing young plants of *Zea mays*, all of the xylem vessels possess annular or spiral secondary walls and that these vessels are extremely large. All of the xylem in the species with which Stover worked was primary in origin.

In *Jatropha cordata*, it has been noted that the first xylem cells to differentiate in the stem are also the first to mature. All of the xylem is secondary in origin. In the hypocotyl, the first xylem cells to differentiate are the first to mature. Most of the xylem in the hypocotyl is secondary although a few primary elements may differentiate and mature after a considerable quantity of secondary xylem has matured. The first xylem cells to differentiate in the roots are located in the center of the root and are primary in origin. The last cells of the primary xylem to differentiate (lying nearest the pericycle) are the first cells of the primary xylem to mature. Secondary xylem forms after all of the primary xylem has differentiated and after much of it has matured. The cells of the xylem with annular and spirally thickened secondary walls were always found to be the first cells to mature although these cells were secondary in origin in the stem and hypocotyl and primary in origin in the root.

The data cited above are conclusive evidence that in the differentiation of xylem cells, the size, location, time of enlargement, time of secondary wall lignification, time of differentiation, and the pattern of the secondary wall do not always bear a specific or constant relationship to the kind of origin, whether primary or secondary. In view of the existing confusion, it seems that the most practical solution of the problem is to discard entirely the terms proto- and metaxylem in

TABLE I

DIRECTION, PLACE, AND TIME OF TISSUE DIFFERENTIATION IN THE ROOT, HYPOCOTYL, AND STEM

Tissue	Organ	Direction of Differentiation	Place and/or Time of Differentiation
Epidermis.....	Root	A	370 μ from root apex; prior to planting
	Hypo.	?	Prior to planting
	Stem	A	10 μ from stem apex, at base of apical meristem; within first 4 days
Hypodermis.....	Root	A	370 μ from root apex; within first 4 days
	Hypo.	?	Prior to planting
	Stem	A	10 μ from stem apex, at base of apical meristem; within first 4 days
Hypodermal cambium..	Root	N	None
	Hypo.	A	Base of hypocotyl; between fourth and seventh days
Cortical parenchyma...	Stem	A	Base of stem; between first and third weeks
	Root	A	320 μ from root apex; prior to planting
	Hypo.	?	Prior to planting
	Stem	A	10 μ from stem apex, at base of apical meristem; within first 4 days
Endodermis.....	Root	A	320 μ from root apex; prior to planting
	Hypo.	N	None
Pericycle.....	Stem	N	None
	Root	A	320 μ from root apex; prior to planting
Pericyclic cambium....	Hypo.	N	None
	Stem	N	None
	Root	A	400 μ from root apex; between fourth and seventh days
Primary phloem.....	Hypo.	N	None
	Stem	N	None
	Root	A	380 μ from root apex; prior to planting
Vascular cambium.....	Hypo.	?	Prior to planting
	Stem	B (?)	Fourth node below stem apex; after fourth day
	Root	A	1600 μ from root apex; within first four days
	(pri)		
	Root	A	At collet; between first and third weeks
	(sec)		
	Hypo.	B (?)	Prior to planting
	(fas)		
	Hypo.	A	Base of hypocotyl; between fourth and seventh days
	(int)		
	Stem	B	20 μ from stem apex, at third node from stem apex; after fourth day
	(fas)		
	Stem	A	At internode elongation; after fourth day
	(int)		
Primary xylem.....	Root	A	310 μ from root apex; prior to planting
	Hypo.	A	Within first 4 days
Pith.....	Stem	N	None
	Root	A	300 μ from root apex; prior to planting
	Hypo.	?	?
	Stem	A	60 μ from stem apex, at fourth node from stem apex; within first 4 days

A, acropetal; B, basipetal; N, none differentiated; pri, primary tissue; sec., secondary tissue; fas, fascicular; int, interfascicular.

favor of meaningful descriptive phrases such as "the first xylem cells to differentiate," "the first xylem cells to mature," or "the xylem elements with spiral, annular, reticulate, or scalariform thickenings," whichever is applicable to the case at hand. It will, of course, be necessary to make clear whether the cells are primary or secondary in origin.

SUMMARY

1. The time and place of origin of the various tissues of the root, hypocotyl, and stem are summarized in Table I. It is apparent that a high degree of tissue differentiation has already taken place in the root primordia and hypocotyl of the embryo by the time of seed maturity, while very little has occurred in the epicotyl.
2. The relative quantities of the various tissues present at different times during the enlargement of the root, hypocotyl, and stem are summarized in figures 1-4.
3. The order of tissue differentiation in the root is (1) pith, (2) primary xylem nearest the center of the root, (3) pericycle, endodermis, and the inner boundary of the cortical parenchyma, (4) hypodermis and epidermis, (5) primary phloem, (6) primary xylem nearest the pericycle, (7) the primary vascular cambium and secondary xylem and phloem, (8) the pericyclic (cork) cambium, and (9) the portions of secondary cambium and tertiary xylem and phloem.

The order of tissue differentiation in the hypocotyl is (1) epidermis, hypodermis, cortical parenchyma, primary phloem (both fascicular and interfascicular), primary fascicular cambium, and pith, (2) secondary fascicular xylem, (3) secondary fascicular phloem and primary xylem, (4) primary interfascicular cambium and secondary interfascicular xylem and phloem.

The order of tissue differentiation in the stem is (1) epidermis, hypodermis, and cortical parenchyma, (2) pith, (3) primary fascicular cambium and secondary xylem, (4) primary phloem, (5) secondary fascicular phloem, (6) primary interfascicular cambium and secondary interfascicular xylem and phloem.

4. The hypocotyl cannot be considered a portion of the stem occupied by the transition region but should be thought of as an organ of the plant possessing a developmental anatomy peculiar to it alone.
5. Elongation of the hypocotyl is due primarily to cell elongation rather than cell division.
6. The phenomena accounting for the extreme enlargement at the base of the hypocotyl are, (a) very little cell elongation accompanies cell enlargement in the base of the hypocotyl while in the upper parts, cell elongation is extreme and accounts for most of cell enlargement, (b) the interfascicular cambium is initiated in the base of the hypocotyl, (c) cambial initials become larger in the base of the hypocotyl and finally divide anticlinally, increasing the total number of cambial cells, (d) reinitiation of intercalary cell divisions throughout the cortex of the hypocotyl commences in the basal end.
7. In the root, a diarch primary xylem is differentiated. The cells nearest the pith differentiate first while the cells nearest the pericycle mature first. In the hypocotyl, an extremely small amount of primary xylem is differentiated after some secondary xylem has formed. No primary xylem is differentiated in the stem.
8. Fibers with thick, cellulose walls comprise more than half of the xylem throughout the mature plant.
9. In the root, during the second or third week, secondary cambia differentiate

across the secondary tissue opposite the xylem points and from it arise tertiary xylem and phloem.

10. Secondary root primordia become initiated in the pericycle opposite the points of the diarch xylem toward the end of the first week.

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